Cont a4

DNA was eluted by adding 0.1 ml of elution buffer (1 mM EDTA, 0.1 N NaOH), incubating 5 min at room temperature, beads were captured with a magnet and the supernatant removed and saved in a new tube. 22.5 μl of precipitation mix containing carrier and pH neutralizers was added along with 2.5 volumes of ethanol. The plasmid DNA was concentrated by centrifugation and re-dissolved in H_2O. Plasmid DNA was re-introduced into E. coli DH10B/P3 by electroporation and selected on LB-agar plates containing 7.5 μg/ml tetracycline and 25 μg/ml ampicillin. Colonies were lifted onto Nytran filters and hybridized with ^{32}P-labeled oligonucleotides with the sequence CAGCTATGGTGGTGCCGACTACAA (SEQ ID NO: 5), AGGTGCTAGGGGACAGTGTTAGACA (SEQ ID NO: 6) , and TCGCTTGTAGTCGGCACCACCATA (SEQ ID NO: 9). All oligos are from AA292201 sequence. Final wash conditions were 2 X SSC, 0.1 % SDS at 55°C for 20 min. The two hybridizing colonies were picked and the sequence of the cDNA inserts was determined.

In the Sequence Listing:

Please add the Sequence Listing pages 1-11 to the application.

REMARKS

The specification has been amended to include a claim of priority to U.S. application serial number 09/644,934 under 35 USC §119, to correct minor informalities and to include the necessary sequence identifiers to comply with 37 CFR §1.821(d). No new matter has been added by way of the amendments to the specification.

In compliance with 37 CFR §1.821(e), 1.821 (f), 1.821(g), and 1.825(b), Applicants submit a Sequence Listing, and a copy of the Sequence Listing in computer readable form (diskette) for the instant application. The Sequence Listing and the computer readable copy are identical in substance and contain no new matter. The computer readable form of the Sequence Listing contained on the enclosed diskette is understood to comply with the requirements of 37 CFR §1.824(d).

SUMMARY

Applicants respectfully request consideration of the above application. If a telephone conversation with Applicants' Attorney would expedite the prosecution of the above-identified application, the examiner is urged to call Applicants' Attorney at (617) 227-7400.

Respectfully submitted,

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Dated: November 2, 2001

APPENDIX A

Version with markings to show changes made to original paragraph 1, page 1:

This application is a divisional application of U.S. application serial number 09/644,934, filed August 23, 2000, pending, which claims priority to U.S. provisional application serial number 60/1509,390, filed on August 23, 1999, both of which are incorporated herein in their [its] entirety by this reference.

Version with markings to show changes made to original paragraphs 1-7 under the heading "Brief Description of the Drawings", Page 7:

Figure 1 depicts the nucleotide sequence encoding a human secreted B7-4, B7-4S (SEQ ID NO: 1).

Figure 2 depicts the nucleotide sequence encoding a human B7-4, B7-4M (SEQ ID NO: 3).

Figure 3 depicts the amino acid sequence of human B7-4S (SEQ ID NO: 2) and illustrates the signal, IgV, IgC, and hydrophilic tail domains.

Figure 4 depicts the amino acid sequence of human B7-4M (SEQ ID NO: 4) and illustrates the signal, IgV, IgC, and transmembrane and cytoplasmic domains.

Figure 5 depicts the nucleotide sequence of murine B7-4 (SEQ ID NO: 10).

Figure 6 depicts the amino acid sequence of murine B7-4 (SEQ ID NO: 11).

Figure 7 depicts an alignment of the human B7-4M (SEQ ID NO: 4) and murine B7-4 (SEQ ID NO: 11) amino acid sequences. Identical residues are reiterated between the two sequences.

Version with markings to show changes made to original paragraph 2, page 96:

Oligonucleotides with the sequence CAGCTATGGTGGTGCCGACTACAA (SEQ ID NO: 5) and AGGTGCTAGGGGACAGTGTTAGACA (SEQ ID NO: 6) from these ESTs were synthesized. These oligonucleotides were used to prime a PCR reaction using as template cDNA

prepared by reverse transcription of mRNAs from the spleen of a case of follicular lymphoma, activated B cells, INF-γ activated keratinocytes, normal spleen, and placenta. Conditions were 94°C, 1 min; 94°C, 30 sec, 56°C, 30 sec, 68°C, 1 min for 35 cycles; 68°C, 3 min, hold 4°C. All templates gave a band of the expected size of 389 bp. The 389 bp product from the PCR of INF-γ activated keratinocytes was purified by agarose gel electrophoresis and 0.12 ng was used as a template in a PCR reaction containing 0.05 mM biotin-21-dUTP and the above primers. Conditions were 94°C, 1 min; 94°C, 30 sec, 56°C, 30 sec, 68°C, 2 min for 20 cycles; 68°C, 5 min, hold 4°C. The biotinylated PCR product was purified on a Nucleospin column (Clontech) and used as a probe in the ClonCapture cDNA selection procedure (Clontech). 60 ng of denatured, biotinylated PCR product was incubated with 2 mM $CoCl_2$, 1 X RecA buffer, 1 μg of RecA protein, 1X ATP in a final volume of 30 μl. The reaction was incubated at 37° for 15 min. 0.7 μg of plasmid DNA of an activated keratinocyte cDNA library and 0.4 μg of a human placental cDNA library was added and incubation continued for 20 min. 50 ng of EcoRV digested lambda DNA was added to the reaction and incubated 5 min. 0.6 μl of 10% SDS and 5.6 μg of proteinase K were added and incubated at 37° for 10 min. Proteinase K was inactivated by adding 1 μl of 0.1 M PMSF. Streptavidin magnetic beads were preincubated with 5 μg of sheared salmon sperm DNA for 10 min and the beads

Version with markings to show changes made to original paragraph 1, of page 97:

captured with a magnet, the supernatant removed, and the beads resuspended in 30 μl of binding buffer (1 mM EDTA, 1 M NaCl, 10 mM Tris-HCl, pH 7.5). The beads were added to the reaction and the reaction incubated for 30 min at room temperature with gentle mixing. The beads were captured with a magnet and the supernatant removed. The beads were washed with 1 ml of washing buffer (1 mM EDTA, 2 M NaCl, 10 mM Tris-HCl, pH 7.5), beads were captured with a magnet and the supernatant removed. The wash procedure was repeated 3 times. One ml of sterile H_2O was added to the washed beads, incubated 5 min at 37°, beads were captured on a magnet and the supernatant removed. Captured DNA was eluted by adding 0.1 ml of elution buffer (1 mM EDTA, 0.1 N NaOH)[.], incubating 5 min at room temperature, beads were

captured with a magnet and the supernatant removed and saved in a new tube. 22.5 μl of precipitation mix containing carrier and pH neutralizers was added along with 2.5 volumes of ethanol. The plasmid DNA was concentrated by centrifugation and re-dissolved in H_2O. Plasmid DNA was re-introduced into E. coli DH10B/P3 by electroporation and selected on LB-agar plates containing 7.5 μg/ml tetracycline and 25 μg/ml ampicillin. Colonies were lifted onto Nytran filters and hybridized with ^{32}P-labeled oligonucleotides with the sequence CAGCTATGGTGGTGCCGACTACAA (SEQ ID NO: 5), AGGTGCTAGGGGACAGTGTTAGACA (SEQ ID NO: 6) , and TCGCTTGTAGTCGGCACCACCATA (SEQ ID NO: 9). All oligos are from AA292201 sequence. Final wash conditions were 2 X SSC, 0.1 % SDS at 55°C for 20 min. The two hybridizing colonies were picked and the sequence of the cDNA inserts was determined.